The role of phosphodiesterase 4 in excessive daytime sleepiness in Parkinson's disease

Heather Wilson,[1] Gennaro Pagano,[1] Flavia Niccolini,[1] Nils Muhlert,[2,3] Mitul A. Mehta,[5] Graham Searle,[4] Roger N. Gunn,[4,6] Eugenii A. Rabiner,[4,5] Thomas Foltynie,[7] and Marios Politis[1]

[1]Neurodegeneration Imaging Group, Institute of Psychiatry, Psychology & Neuroscience (IoPPN), King's College London, United Kingdom
[2]School of Psychology and Cardiff University Brain Research Imaging Centre, Cardiff University, United Kingdom
[3]Division of Neuroscience & Experimental Psychology, University of Manchester, UK
[4] Invicro London, Hammersmith Hospital, London, United Kingdom
[5] Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, United Kingdom
[6]Division of Brain Sciences, Imperial College London, Hammersmith Hospital, London, United Kingdom
[7] Sobell Department of Motor Neuroscience, UCL Institute of Neurology, London, United Kingdom

Correspondance to: Professor Marios Politis, Neurodegeneration Imaging Group, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, 125 Coldharbour Lane, Camberwell, London SE5 9NU, UK. E-mail: marios.politis@kcl.ac.uk

Word count: Title=11; Abstract=234; Manuscript=2,940
No. of References: 32
Figures/Tables: 1/3

Keywords: Parkinson's disease, Phosphodiesterase 4, Positron Emission Tomography, Diffusion Tensor Imaging, Rolipram, Excessive Daytime Sleepiness

Conflict of interest declaration: The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Ethical standard: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review boards and the local research ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained for all human participants.

Author Contributions: M.P. conceived the study, conceptualized the experimental design and acquired funding for the study. F.N., H.W., E.A.R., R.N.G., T.F. and M.A.M gave input to experimental design. F.N. and G.P performed the imaging and clinical assessments and acquired the data. F.N., G.P., E.A.R. and M.P. organised the study. H.W. wrote the first draft and prepared the manuscript. H.W. generated the figures. H.W. and G.P. analysed the data. H.W. and M.P., interpreted the data. F.N., T.F. and G.P. recruited the subjects. All authors revised and gave input to the manuscript.

Acknowledgments: We thank all participants and their families, the PET technicians and radiochemists, the MRI radiographers, and the clinical research nurses at Invicro London for their cooperation and support with this study.

Funding: This study represents independent research supported by the Edmond J. Safra Foundation and the National Institute for Health Research (NIHR) / Wellcome Trust King's Clinical Research Facility and the NIHR Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. Tom Foltynie's research is supported by the Brain Research Trust, UCL's NIHR Biomedical Research Unit, the European Union FP-7 scheme and the Michael J. Fox Foundation. Marios Politis research is supported by Parkinson's UK, Lily and Edmond J. Safra Foundation, Michael J Fox Foundation (MJFF) for Parkinson's research, KCL's NIHR Biomedical Research Unit, and The CHDI Foundation.

Abstract

Introduction: Preclinical studies suggest a link between cAMP/PKA signalling, phosphodiesterase 4 (PDE4) expression and excessive daytime sleepiness (EDS). Here, we investigated *in vivo* the association between PDE4 expression and EDS in Parkinson's disease (PD) patients using [^{11}C]rolipram PET and MR imaging.

Methods: Eighteen participants, 12 PD and 6 healthy controls, underwent one [^{11}C]rolipram PET and a multi-modal MRI scan. Probabilistic tractography was performed on subjects' diffusion data to functionally parcellate the striatum according with projections to limbic cortical areas. The severity of EDS was assessed using the Epworth Sleepiness Scale (ESS). To assess PDE4 expression in PD patients with EDS, the PD cohort was divided according to the presence (n=5) or absence (n=7) of EDS, defined using validated cut-off of score ≥10 on the ESS as score ≥10 on the ESS.

Results: PD patients with EDS showed significantly increased [^{11}C]rolipram volume of distribution (V_T) in the caudate (*P*=0.029), hypothalamus (*P*=0.013), hippocampus (*P*=0.036) and limbic striatum (*P*=0.030) compared to patients without EDS. Furthermore, higher ESS scores correlated with increased [^{11}C]rolipram V_T in the caudate (*r*=0.77; *P*=0.003), hypothalamus (*r*=0.84; *P*=0.001), hippocampus (*r*=0.81; *P*=0.001) and limbic subdivisions of the striatum (*r*=0.80; *P*=0.003).

Conclusion: Our findings translate into humans preclinical data indicating that EDS is associated with elevated PDE4 in regions regulating sleep. The severity of EDS in PD was associated with elevated PDE4 expression; thus, suggesting a role of PDE4 in the pathophysiology of EDS in PD.

Introduction

Excessive daytime sleepiness (EDS) is one of the most common sleep disturbances in Parkinson's disease (PD) affecting around 50% of patients [1]. EDS is characterised by a tendency of rapid sleep onset during waking hours without prior drowsiness [2] and has been linked to deficits in attention and memory [3]. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies have shown that dopaminergic and serotonergic dysfunction may contribute to the development of EDS in PD [4-6]. However, the mechanisms underlying pathophysiology of daytime sleepiness in PD remain unclear.

Preclinical studies have provided evidence that phosphodiesterase 4 (PDE4) plays a role in daytime sleepiness [7-10]. PDE4 is an intracellular enzyme which hydrolyses cyclic adenosine monophosphate (cAMP), thus regulating signalling cascade including cAMP-protein kinase A (PKA)-cAMP response binding protein (CREB) [11]. PDE4 is highly expressed in the striatum, thalamus, cerebellum, hippocampus and cortex [12]. A genome-wide association study of sleep and circadian phenotypes identified eight single nucleotide polymorphism (SNP) were associated with sleep phenotypes [10]. The SNP rs1823068, located in an intron of the PDE4 gene on chromosome 5, had the strongest association with increased daytime sleepiness [10]. Elevated cAMP/PKA signalling has recently been reported in the brain of an animal model of Huntington's disease (HD) animal models with sleep deficits [8]. Moreover, selective PDE4 inhibitors promote wakefulness [7]. Sleep deprivation has also been shown to increase PDE4 protein levels and activity in the hippocampus, subsequently reducing cAMP signalling and impairing hippocampal cAMP/PKA-dependent forms of synaptic plasticity [9].

A recent PET study showed altered PDE4 expression in Parkinson's disease patients [13]. Therefore, given the preclinical evidence for the role of PDE4 in sleep-related problems we aimed to investigate whether changes in PDE4 levels could be related to the development of

EDS. We hypothesized that elevated PDE4 levels, in brain regions regulating the sleep-wake cycle, could play a role in the pathophysiology of EDS in PD. Here, we investigated *in vivo* the expression of PDE4 in PD patients with and without EDS using multi-modal magnetic resonance imaging (MRI) and PET with [^{11}C]rolipram, a selective PDE4 radioligand [14, 15].

Methods

Participants

We recruited eighteen subjects, twelve patients with idiopathic PD and six age- and gender-matched healthy controls with no history of any psychiatric or neurological disorders and not on treatment with medications. PD patients had no history of other neurological or psychiatric disorders, and were not under treatment with substances with known actions in PDEs. All PD patients were on stable dopaminergic treatment (levodopa and/or dopamine agonists) for at least six months at the time of study enrolment. Daily dopamine agonist equivalent dose (LED_{DA}) and daily levodopa equivalent dose ($LED_{L\text{-}DOPA}$) unit calculations were based on theoretical equivalence to levodopa as described previously [16]. Participants underwent clinical assessments to evaluate motor, cognitive and psychiatric symptoms severity (Table 1). Motor symptom severity was assessed with the Unified Parkinson's Disease Rating Scale part-III (UPDRS-III) and staged with Hoehn & Yahr (H&Y) scale. Motor assessments were performed OFF medication after overnight withdrawal of patient's dopaminergic medications and following a challenge with levodopa 250/carbidopa 25, with an observational ON-medication period of 150 min. Quality of life was measured with the patient self-reported 39-item Parkinson's disease Questionnaire (PDQ-39). Non-motor symptoms were assessed using UPDRS part-I (UPDRS-I), Non-Motor symptom scale (NMSS), Parkinson's disease sleep scale (PDSS) and Epworth Sleepiness Scale (ESS). Depression was assessed using the Beck

Depression Inventory-II (BDI-II) and Hamilton Depression Rating Scale (HAM-D). General cognitive status was assessed using the Mini Mental Status Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

Severity of EDS was assessed using the Epworth Sleepiness Scale (ESS); higher ESS scores indicate greater daytime sleepiness. To assess PDE4 expression in PD patients with EDS, the PD cohort was divided according to the presence (n=5) or absence (n=7) of EDS, defined using validated cut-off of score ≥10 on the ESS as score ≥10 on the ESS [17] (Table 1).

All participants screened successfully to undertake PET and MRI scanning under standard criteria (http://www.mrisafety.com; https://www.gov.uk/government/publications/arsac-notes-for-guidance). Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki. The study was approved by the institutional review boards and the research ethics committee.

Scanning procedures

PET and MR imaging was performed at Invicro, London, UK. Patients stopped all dopaminergic medication and withheld from consumption of caffeinate beverages for 12 hours before the scan. Long acting dopaminergic medications were withdrawn for 72 hours prior to the PET scan. MRI scans were acquired with a 32-channel head coil on a Siemens Magnetom Verio 3-T MRI scanner and included: T_1-weighted magnetization prepared rapid acquisition with gradient echo sequence (MPRAGE; time repetition (TR) = 2300ms, time echo (TE) = 2.98ms, flip angle of 9°, time to inversion (T1) = 900ms, matrix = 240 x 256) for co-registration with the PET images; fast grey matter T_1 inversion recovery (FGATIR; TR = 6000ms, TE = 2.96ms, flip angle of 8°, T1 = 409ms, matrix = 240 x 256) for manual delineation of subcortical regions of interest; and diffusion-weighted data (DTI) for probabilistic tractography and

connectivity-based parcellation of the striatum. All sequences used a 1 mm^3 voxel size, anteroposterior phase encoding direction, and a symmetric echo.

Diffusion-weighted data were acquired for performing probabilistic tractography and connectivity-based parcellation of the striatum using echo planar imaging (EPI; TR = 8000ms, TE = 96ms, flip angle of 90° and voxel size of 2 x 2 x 2 mm^3). The diffusion weighting as isotropically distributed along the 32 directions (b-value = 1000 s/mm2), and a non-diffusion-weighted imaging (b0) was acquired at the beginning of each scan. To minimize EPI geometric distortions, two image sets were acquired with the phase-encoded direction reversed, 'blip-up' and 'blip-down', allowing for the calculation of a corrected image.

[^{11}C]Rolipram, PET scans were obtained on a Biograph Hi-Rez 6 PET-CT scanner (Siemens) one hour following a standard levodopa 250/carbidopa 25 dose. A mean dose of 306.0 MBq [^{11}C]rolipram (SD: ± 49.0) [mean mass injected: 2.8 ug (SD: ± 1.6)] was intravenously administered as a slow bolus injection over 20s. Dynamic emission data were acquired continuously for 90 minutes. The dynamic images were reconstructed into 26 frames using a filter back projection algorithm (direction inversion Fourier transform) with a 128 matric, zoom of 2.6 producing images with isotropic voxel size 2 x 2 x 2 mm^3, and smoothed with a transaxial Guassian filter of 5mm. Blood sampling was performed through an arterial line inserted in the radial artery to generate arterial plasma input data. For the initial 15 minutes radioactivity levels in blood was continuously measured through an automatic blood sampling system at 5ml/min, followed by samples at 5, 10, 15, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 min during the scan. [^{11}C]rolipram parent fraction over the course of the PET scan was determined by HPLC using the Hilton column switching method [18].

Imaging data analysis

MRI-based volumetric analysis

Grey matter atrophy has been reported in PD patients with EDS compared to those with EDS [19]. Therefore, we investigated volumetric changes in cortical and subcortical nuclei regions between groups of PD patients using the FreeSurfer's image analysis suite (version 5.3.0 http://surfer.nmr.mgh.harvard.edu) to process individual MRI scans for deriving measures of cortical thickness and subcortical nuclei volumes. Adjustments for intracranial volume were calculated for each region-of-interest (ROI) using validated methods within the FreeSurfer toolkit. We also carried out volumetric analysis, on each subjects volumetric T1-weighted MRI, for bilateral manually delineated ROIs and DTI connectivity-based ROIs.

[^{11}C]rolipram PET data analysis

Image processing and kinetic modelling was carried out using the Molecular Imaging and Kinetic Analysis Toolbox software package (MIAKAT^{TM}: www.miakat.org), implemented in MATLAB (The Mathworks, Natick, MA, USA). MIAKAT^{TM} combines in-house code with wrappers for FMRIB Software Library (FSL, University of Oxford) and Statistical Parametric Mapping (SPM, Wellcome Trust Centre for Neuroimaging). For [^{11}C]rolipram data, parent in plasma input function were generated using the continuous and discrete blood samples. The Hill model was used to model the parent fraction data [20]. Regional time-activity data was derived from dynamic PET data and volume of distribution (V_T) values estimated using the 2-Tissue compartmental model. Parametric [^{11}C]rolipram V_T images were generated with the Logan plot [21].

Region-of-interest analysis

To investigate whether changes in PDE4 levels could be related to the development of EDS, we quantified PDE4 levels, in brain regions involved in the limbic system and in regulating the sleep-wake cycle. Parametric PET images were co-registered and resliced to corresponding FGATIR MR images in SPM12 software package implemented in Matlab 2015a. The

anatomical CIC atlas version 2.0 [22] was used to define allo-cortical ROIs involved in the limbic system including the amygdala, hippocampus, orbitofrontal, cingulate and temporal cortex. Subcortical ROIs, including the ventral striatum, caudate, putamen, thalamus, hypothalamus, dorsal and ventral raphe, were manually delineated on subject's FGATIR MR images using Analyze version 12 (Mayo Foundation) medical imaging software package following published guidelines [22]. PDE4 levels were also quantified in additional brain regions, defined using the CIC atlas v2.0, not involved in regulating the sleep-wake cycle, please see supplemental materials Table S1.

Connectivity-based parcellations according to cortico-striatal projections

Diffusion data analysis was performed using FSL Diffusion Toolbox (FDT) (FMRIB Centre Software Library, Oxford University). Each phase encoding direction image set, blip-up and blip-down, was corrected for motion and eddy current-related distortions. FDT software was used to perform probabilistic tractography on each subjects' diffusion data to parcellate the striatum according projections to cortical limbic regions [23, 24]. Methods were following as previously described [23, 24]. For each striatal voxel 10,000 sample tracts were generated to enable estimates of striatal connectivity profile with each target. The cortical-striatal connectivity maps were thresholded at 5% of the maximum connectivity value to minimise noise and voxels with low connectivity values, allowing functional subdivisions to have a certain degree of overlap. The resulting output connectivity maps for functional subdivisions of the striatum according to connectivity to limbic cortical areas were transformed to subjects' structural T1 space. Output connectivity maps were applied to parametric [^{11}C]rolipram V_T images to calculate regional estimates of V_T. The topography of the limbic striatum according to connectivity to the cortical limbic regions is illustrated in Figure 1.

Statistical analysis

Statistical analysis and graph illustration were performed with SPSS (version 20 Chicago, Illinois, USA) and GraphPad Prism (version 6.0) respectively. For all variables, variance homogeneity and normality were tested with Bartlett and Kolmogorov-Smirnov tests. Independent t-test (parametric variables) and Mann-Whitney U test (non-parametric variables) were used for between-group comparisons, as appropriate, and P values were calculated following Bonferroni's multiple comparisons accounting for the number of regions in allo-cortical and subcortical groups. Analysis of covariance (ANCOVA) was used to covariate for age, disease duration, motor symptom severity, LED_{DA}, $LED_{L\text{-}DOPA}$, depression and cognitive status in between groups comparisons. We interrogated correlations between PET and clinical data using Spearman's *r* correlation coefficient and applied Benjamini-Hochberg correction with the false discovery rate cut-off at 0.05. Correlations were repeated including age, disease duration, motor symptom severity, LED_{DA}, $LED_{L\text{-}DOPA}$, depression and cognitive status as covariate. All data are presented as mean±SD, and the level α was set for all comparisons at $P<0.05$, Benjamini-Hochberg corrected.

Results

No differences were found in clinical measures including age ($P>0.10$), disease duration ($P>0.10$), motor symptoms severity (P>0.10), daily intake of total levodopa equivalent units (LED_{Total}; $P>0.10$), daily LED_{DA} (P>0.10) or daily $LED_{L\text{-}DOPA}$ (P>0.10), depressive symptoms [BDI-II ($P>0.10$), HAM-D ($P>0.10$)] and cognitive function [MMSE ($P>0.10$); MoCA ($P>0.10$)] between the group of PD patients with and without EDS (Table 1).

PD patients with EDS had significantly increased [^{11}C]rolipram V_T compared to patients without EDS but not compared to healthy controls (Figure 2B). Specifically, [^{11}C]rolipram V_T was significantly increased in PD patients with EDS in the caudate ($P=0.029$), hypothalamus

(*P*=0.013), hippocampus (*P*=0.036) and limbic striatum (*P*=0.030) compared to PD without EDS (Figure 2A, Table 2). The results remained consistent after covariation for age, disease duration, motor symptom severity, LED_{DA}, $LED_{L\text{-}DOPA}$ depression and cognitive status. MRI volumetric analysis confirmed no significant differences in ROIs volume between groups (*P*>0.05) (Table 3).

To support findings that PDE4 levels are elevated in brain regions regulating the sleep-wake cycle, we also quantified PDE4 levels in additional brain regions not involved in sleep regulation. We found no differences in frontal, occipital, temporal and parietal regions between PD patients with and without EDS (Table S1).

Higher ESS scores were associated with increased [^{11}C]rolipram V_T in the caudate (*r*=0.77; *P*=0.003), hypothalamus (*r*=0.84; *P*=0.001), hippocampus (*r*=0.81; *P*=0.001) and limbic subdivisions of the striatum (*r*=0.80; *P*=0.003) (Figure 2C). These results were confirmed after controlling for age, disease duration, motor symptom severity, LED_{DA}, $LED_{L\text{-}DOPA}$ depression and cognitive status.

Discussion

Our findings suggest that EDS in PD is associated with increased PDE4 expression in brain regions involved in the regulation of sleep and wakefulness. We used a region-of-interest based approach, combining anatomical and DTI connectivity-based analysis, to assess PDE4 expression in the caudate, hypothalamus, hippocampus and limbic subdivisions of the striatum. Our findings translate to humans, preclinical data, which have demonstrated that elevated levels of PDE4 expression are involved in the pathophysiology of EDS [7, 9, 10].

Recently, loss of PDE4 expression has been reported in PD patients compared to healthy controls, with decreased PDE4 associated with deficits in spatial working memory [13]. Here, we report relative increased PDE4 expression associated with severity of EDS in limbic brain regions. Elevated PDE4, in specific brain regions involved in sleep regulation, occurs only in PD with EDS and is evident when comparing similar disease stage and cognitive status PD patients without EDS but not when compared to healthy controls. In PD with EDS, relative increases in PDE4 might mask PD-related loss of PDE4 and conversely, increased PDE4 levels might be underestimated due to parallel loss of PDE4. It could be argued that substantial increased PDE4 expression would have to occur in order to counter the PD-related loss of PDE4, and is necessary for development of EDS. Further studies in larger cohorts are warranted to elucidate the relationship between overall PD-related loss of PDE4 and regional increased in PDE4 associated with EDS.

The hypothalamus is a critical area involved in modulating the sleep-wake cycle; the ventrolateral preoptic nucleus acts to inhibit major arousal mechanisms, thus promoting sleep [25]. Recently, the severity of EDS in PD was shown to be associated with reduced hypothalamic post-synaptic dopamine type-3 receptor (D_3R) availability [5], as well as striatal dopaminergic dysfunction [4]. PDE4 regulates cAMP/PKA signalling at dopaminergic terminals subsequently modulating dopamine activity [11]. Therefore, by modulating dopamine release, elevated PDE4 levels in dopaminergic innervating neurons to the hypothalamus and striatum could influence dopamine receptor availability contributing to mechanisms underlying EDS. *In vitro* and animal studies showed that rolipram, a PDE4 inhibitor, can increase dopaminergic tone in the striatum by stimulating dopamine synthesis and decreasing striatal D_2R signalling [11, 26]. Therefore, elevated PDE4 could promote daytime sleepiness *via* modulating striatal dopaminergic tone and dopamine receptor signalling [27, 28]. Furthermore, elevated PDE4 levels in PD with EDS could exacerbate striatal

dopaminergic degeneration by decreasing CREB phosphorylation [29, 30]. A recent preclinical study demonstrated that elevated cAMP/PKA signalling is linked to sleep dysfunction [8]. Further studies are required to understand if increases in PDE4 expression are a compensatory mechanism to counteract earlier increase in cAMP/PKA signalling, or if elevated PDE4 is a primary pathological process underlying sleep dysfunction in PD.

DTI connectivity-based analysis revealed increases in PDE4 expression within limbic subdivisions of the striatum, which was associated with the severity of daytime sleepiness. During sleep the limbic system is thought to act to integrate emotions and memory, and consolidating memories [31]. Behavioural changes due to sleep deprivation, such as cognitive deficits and mood changes [32], have been linked to increased PDE4 expression and altered cAMP signalling [32]. In a preclinical study, sleep deprivation selectively impaired cAMP/PKA-dependent forms of synaptic plasticity in the hippocampus, through increased activity and protein levels of PDE4 [9]. Treatment with the PDE4 inhibitor rolipram, rescued sleep deprivation-induced deficits in cAMP signalling, synaptic plasticity and hippocampus-dependent memory [9]. Furthermore, a preclinical animal study demonstrated that rolipram was able to enhance wakefulness by increasing the availability of cAMP [7]. Here, we suggest that relatively increased PDE4 expression occur only in PD patients with EDS. Therefore, modulation of PDE4 in PD with EDS, to restore to normal levels, could be a suitable pharmacological target to ameliorate daytime sleepiness, as well as behavioural changes related to sleep deprivation. However, further studies in larger cohorts of patients and healthy controls are required to confirm these results.

In this study, there were no differences in the dopaminergic medication, LED_{DA} or $LED_{L\text{-}DOPA}$, between groups of PD patients with and without EDS suggesting that the upregulation of PDE4 in PD patients with EDS is not driven by dopaminergic medication. However, it is worth considering that the absence of association between the use of dopamine agonist and the

upregulation of PDE4 does exclude the possibility that dopamine agonists could induce EDS and the upregulation of PDE4. Further studies are warranted to fully elucidate the relationship between dopamine medication, in particular dopamine agonists, and the upregulation of PDE4 in association with EDS. A limitation of the current study is the small sample sizes which might reduce statistical power to detect between group differences in clinical variables which might influence PDE4 levels. Therefore, while our results remained after controlled for clinical variables, we cannot completely exclude the potential influence of other factors on PDE4 levels. Further studies with larger cohorts of PD patients with and without EDS are required to validate our findings.

In conclusion, we provide preliminary evidence that elevated PDE4 expression is associated with the severity of EDS in PD patients. Therefore, altered PDE4 expression could represent a possible mechanism underlying the pathophysiology of EDS in PD.

Figure legend

Figure 1: Segmentation of the limbic striatum based on cortical-connectivity. Schematic illustration of striatal subdivisions (left) according to cortical-connectivity associated with limbic (orange), cognitive (blue) and sensorimotor (green) functional specialisation. Probabilistic tractography was used to parcellate the striatum according to projections to the limbic cortex to create the limbic striatum (orange). Connectivity maps, of the limbic striatum, were produced for subject where each voxel in the striatum is assigned according to the probability of connectivity to limbic cortical areas. An output connectivity map for a representative healthy control in shown (right) to illustrate topography of the limbic striatum.

Figure 2: Elevated phosphodiesterase 4 (PDE4) in Parkinson's disease (PD) patients with daytime sleepiness. (A) Bar graphs showed increased PDE4 in PD with EDS compared to PD without EDS and healthy controls; (B) [^{11}C]rolirpam PET images for a PD patient with EDS (80 year old female; disease duration 25 years; ESS=14) and a PD patient without EDS (68 year old female; disease duration 7 years; ESS=3); (C) Significant positive correlation between increased [^{11}C]rolipram V_T and higher ESS scores. *P<0.05 Bonferroni corrected for multiple-comparisons. Abbreviations: PDE4 = Phosphodiesterase 4; PD = Parkinson's disease; EDS = Excessive Daytime Sleepiness; EES = Epworth Sleepiness Scale; V_T = Volume of Distribution.

Tables

Table 1: Clinical characteristics of Parkinson's disease patients and healthy controls

	Healthy Controls	**PD without EDS ESS <10**	**PD with EDS ESS ≥10**
No Subjects	6	7	5
Sex	4 M / 2 F	5 M / 2 F	2 M/ 3 F

Age (years ±SD)	64.9 (±3.0)	71.2 (±6.1)	70.8 (±10.0)
Disease duration (years ±SD)	-	9.7 (±4.4)	13.4 (±7.9)
Daily LED_{Total} (mg±SD)	-	1130.0 (±1409.0)	1031.9 (±878.5)
Daily $LED_{L\text{-}DOPA}$ (mg±SD)	-	1047.9 (±1368.0)	891.9 (±848.6)
Daily LED_{DA} (mg±SD)	-	82.14 (±80.3)	140.0 (±128.1)
H&Y (mean ±SD)	-	2.6 (±1.0)	2.9 (±1.0)
UPDRS-III (mean ±SD)	-	34.7 (±15.7)	44.6 (±16.7)
UPDRS-I total (mean ±SD)	2.8 (±3.8)	8.6 (±7.1)	9.5 (±8.1)
NMSS total (mean ±SD)	6.3 (±8.5)	43.9 (±37.0)	61.6 (±18.2)
PDSS (mean ±SD)	126.2 (±26.9)	117.0 (±12.7)	88.2 (±27.2)*
ESS (mean ±SD)	3.3 (±3.3)	5.0 (±2.2)	12.2 (±2.3)***
MMSE (mean ±SD)	29.8 (±0.4)	27.7 (±1.6)	28.00 (±2.4)
MOCA (mean ±SD)	28.2 (±1.6)	27.1 (±2.1)	28.00 (±2.0)
HAM-D (mean ±SD)	1.3 (±1.2)	4.3 (±3.3)	8.6 (±5.2)
BDI-II (mean ±SD)	1.8 (±2.5)	6.4 (±3.7)	12.0 (±7.0)
PDQ-39 (mean ±SD)	0.3 (±0.2)	25.3 (±18.2)	47.2 (±22.1)

Abbreviations: $LED_{L\text{-}DOPA}$ = Levodopa Equivalent Dose; LED_{DA} = Dopamine Agonist Equivalent Dose; H&Y = Hoehn and Yahr; UPDRS = Unified Parkinson's Disease Rating Scale; PDQ-39 = 39-item Parkinson's Disease Questionnaire; MMSE = Mini Mental State Examination; BDI-II = Beck Depression Inventory-II; HAM-D = Hamilton Depression Rating Scale; PDSS = Parkinson's Disease Sleep Scale; EES = Epworth Sleepiness Scale; NMS = Non-Motor Symptoms assessment scale; SD = Standard Deviation. UPDRS scores reported in OFF medication state after overnight withdrawal of patient's dopaminergic medication. Comparisons between PD groups with and without EDS: ***P<0.001; **P<0.05.

Table 2: [^{11}C]Rolipram volume of distribution (V_T) in groups of Parkinson's patients with and without excessive daytime sleepiness

	Healthy Controls (n=6)	**PD without EDS ESS <10 (n=7)**	**PD with EDS ESS >10 (n=5)**	***P* value % change[b]**
Caudate	0.70 (±0.15)	0.53 (±0.17)	0.74 (±0.11)	**0.029 38%**
Putamen	0.86 (±0.15)	0.73 (±0.18)	0.95 (±0.12)	>0.05
Ventral Striatum	0.83 (±0.14)	0.72 (±0.17)	0.93 (±0.10)	>0.05
Thalamus	0.74 (±0.14)	0.57 (±0.16)	0.72 (±0.10)	>0.05
Hypothalamus	0.58 (±0.11)	0.48 (±0.12)	0.66 (±0.06)	**0.013 37.7%**
Dorsal Raphe	0.60 (±0.11)	0.50 (±0.13)	0.64 (±0.07)	>0.05
Ventral Raphe	0.57 (±0.09)	0.47 (±0.12)	0.62 (±0.07)	>0.05
Amygdala	0.73 (±0.15)	0.58 (±0.16)	0.76 (±0.10)	>0.05
Limbic Striatum[a]	0.95 (±0.12)	0.69 (±0.19)	0.92 (±0.09)	**0.030 33.9%**
Hippocampus	0.78 (±0.8)	0.58 (±0.15)	0.76 (±0.07)	**0.036 30.8%**
Orbitofrontal Cortex	0.84 (±0.13)	0.68 (±0.18)	0.87 (±0.10)	>0.05
Cingulate	0.80 (±0.14)	0.63 (±0.16)	0.83 (±0.10)	>0.05
Temporal Cortex	0.82 (±0.09)	0.61 (±0.16)	0.81 (±0.08)	>0.05

[a] Limbic Striatum: functional connectivity-based striatal subdivision connecting to cortical limbic regions. [b]P values (Bonferroni corrected) and percentage change for between Parkinson's disease groups with and without excessive daytime sleepiness. Data shown as (mean±SD). Abbreviations: EDS = Excessive daytime sleepiness; ESS= Epworth Sleepiness Scale; PD = Parkinson's disease.

Table 3: MRI volumetric analysis in healthy controls and Parkinson's disease patients with and without excessive daytime sleepiness

Region	**Healthy Controls**	**PD without EDS ESS <10**	**PD with EDS ESS >10**	***P* value**
Caudate R	2.20 (±0.07)	2.25 (±0.28)	2.37 (±0.34)	>0.05
Caudate L	2.18 (±0.07)	2.14 (±0.29)	2.33 (±0.26)	>0.05

Putamen R	3.09 (±0.26)	2.97 (±0.46)	3.17 (±0.11)	>0.05
Putamen L	3.39 (±0.32)	3.22 (±0.55)	3.52 (±0.29)	>0.05
Ventral Striatum R	1.11 (±0.17)	1.21 (±0.21)	0.93 (±0.34)	>0.05
Ventral Striatum L	1.04 (±0.18)	1.10 (±0.20)	0.90 (±0.18)	>0.05
Thalamus R	4.17 (±0.35)	4.04 (±0.38)	4.04 (±0.45)	>0.05
Thalamus L	4.69 (±0.44)	4.66 (±0.68)	4.57 (±0.43)	>0.05
Hypothalamus R	0.14 (±0.06)	0.16 (±0.06)	0.15 (±0.06)	>0.05
Hypothalamus L	0.16 (±0.06)	0.17 (±0.06)	0.14 (±0.04)	>0.05
Dorsal Raphe	0.30 (±0.12)	0.31 (±0.08)	0.31 (±0.06)	>0.05
Ventral Raphe	0.26 (±0.11)	0.28 (±0.10)	0.28 (±0.04)	>0.05
Hippocampus R	2.66 (±0.21)	2.22 (±0.31)	2.48 (±0.21)	>0.05
Hippocampus L	2.52 (±0.15)	2.19 (±0.37)	2.38 (±0.30)	>0.05
Limbic Striatum R	1.96 (±0.62)	1.50 (±0.60)	1.92 (±0.30)	>0.05
Limbic Striatum L	1.67 (±0.61)	1.69 (±0.54)	1.85 (±0.53)	>0.05
Amygdala R	1.03 (±0.11)	0.80 (±0.26)	0.98 (±0.15)	>0.05
Amygdala L	0.97 (±0.10)	0.84 (±0.24)	0.98 (±0.12)	>0.05
Orbitofrontal Cortex R	2.40 (±0.10)	2.39 (±0.13)	2.38 (±0.23)	>0.05
Orbitofrontal Cortex L	2.51 (±0.08)	2.41 (±0.13)	2.44 (±0.15)	>0.05
Cingulate R	2.35 (±0.14)	2.43 (±0.16)	2.27 (±0.32)	>0.05
Cingulate L	2.52 (±0.17)	2.49 (±0.13)	2.67 (±0.17)	>0.05
Temporal Cortex R	3.65 (±0.32)	3.48 (±0.24)	3.44 (±0.60)	>0.05
Temporal Cortex L	3.57 (±0.24)	3.43 (±0.46)	3.44 (±0.13)	>0.05

Data shown as (mean±SD). Abbreviations: PD = Parkinson's disease; EDS = Excessive Daytime Sleepiness; ESS= Epworth Sleepiness Scale.

References

1. Knie, B., et al., *Excessive daytime sleepiness in patients with Parkinson's disease.* CNS Drugs, 2011. **25**(3): p. 203-12.
2. Frucht, S., et al., *Falling asleep at the wheel: motor vehicle mishaps in persons taking pramipexole and ropinirole.* Neurology, 1999. **52**(9): p. 1908-10.
3. Adler, C.H. and M.J. Thorpy, *Sleep issues in Parkinson's disease.* Neurology, 2005. **64**(12 Suppl 3): p. S12-20.
4. Happe, S., et al., *Association of daytime sleepiness with nigrostriatal dopaminergic degeneration in early Parkinson's disease.* Journal of Neurology, 2007. **254**(8): p. 1037.
5. Pagano, G., et al., *Sleep problems and hypothalamic dopamine D3 receptor availability in Parkinson disease.* Neurology, 2016. **87**(23): p. 2451-2456.
6. Pavese, N., et al., *SLEEP REGULATORY CENTRES DYSFUNCTION IN PARKINSON'S DISEASE PATIENTS WITH EXCESSIVE DAYTIME SLEEPINESS. AN IN VIVO PET STUDY.* Parkinsonism & Related Disorders, 2012. **18**: p. S24-S25.
7. Lelkes, Z., et al., *Rolipram, an antidepressant that increases the availability of cAMP, transiently enhances wakefulness in rats.* Pharmacol Biochem Behav, 1998. **60**(4): p. 835-9.
8. Gonzales, E.D., et al., *Early-onset sleep defects in Drosophila models of Huntington's disease reflect alterations of PKA/CREB signaling.* Hum Mol Genet, 2016. **25**(5): p. 837-52.
9. Vecsey, C.G., et al., *Sleep deprivation impairs cAMP signalling in the hippocampus.* Nature, 2009. **461**(7267): p. 1122-5.
10. Gottlieb, D.J., G.T. O'Connor, and J.B. Wilk, *Genome-wide association of sleep and circadian phenotypes.* BMC Med Genet, 2007. **8 Suppl 1**: p. S9.
11. Nishi, A., et al., *Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum.* Journal of Neuroscience, 2008. **28**(42): p. 10460-10471.
12. Menniti, F.S., W.S. Faraci, and C.J. Schmidt, *Phosphodiesterases in the CNS: targets for drug development.* Nature Reviews Drug Discovery, 2006. **5**(8): p. 660-670.
13. Niccolini, F., et al., *Loss of phosphodiesterase 4 in Parkinson disease: Relevance to cognitive deficits.* Neurology, 2017. **89**(6): p. 586-593.
14. Fujita, M., et al., *Quantification of brain phosphodiesterase 4 in rat with (R)-[11C]Rolipram-PET.* Neuroimage, 2005. **26**(4): p. 1201-10.
15. DaSilva, J.N., et al., *Imaging cAMP-specific phosphodiesterase-4 in human brain with R- C-11 rolipram and positron emission tomography.* European Journal of Nuclear Medicine and Molecular Imaging, 2002. **29**(12): p. 1680-1683.
16. Politis, M., et al., *Staging of serotonergic dysfunction in Parkinson's disease: an in vivo 11C-DASB PET study.* Neurobiol Dis, 2010. **40**(1): p. 216-21.
17. Johns, M.W., *A new method for measuring daytime sleepiness: the Epworth sleepiness scale.* Sleep, 1991. **14**(6): p. 540-5.
18. Hilton, J., et al., *Column-switching HPLC for the analysis of plasma in PET imaging studies.* Nucl Med Biol, 2000. **27**(6): p. 627-30.
19. Kato, S., et al., *Widespread cortical and subcortical brain atrophy in Parkinson's disease with excessive daytime sleepiness.* J Neurol, 2012. **259**(2): p. 318-26.
20. Tonietto, M., et al., *Improved models for plasma radiometabolite correction and their impact on kinetic quantification in PET studies.* J Cereb Blood Flow Metab, 2015. **35**(9): p. 1462-9.
21. Logan, J., et al., *Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl]-(-)-cocaine PET studies in human subjects.* J Cereb Blood Flow Metab, 1990. **10**(5): p. 740-7.

22. Tziortzi, A.C., et al., *Imaging dopamine receptors in humans with C-11 -(+)-PHNO: Dissection of D3 signal and anatomy.* Neuroimage, 2011. **54**(1): p. 264-277.
23. Niccolini, F., et al., *Altered PDE10A expression detectable early before symptomatic onset in Huntington's disease.* Brain, 2015. **138**(Pt 10): p. 3016-29.
24. Tziortzi, A.C., et al., *Connectivity-based functional analysis of dopamine release in the striatum using diffusion-weighted MRI and positron emission tomography.* Cereb Cortex, 2014. **24**(5): p. 1165-77.
25. Saper, C.B., T.C. Chou, and T.E. Scammell, *The sleep switch: hypothalamic control of sleep and wakefulness.* Trends Neurosci, 2001. **24**(12): p. 726-31.
26. Nishi, A. and G.L. Snyder, *Advanced Research on Dopamine Signaling to Develop Drugs for the Treatment of Mental Disorders: Biochemical and Behavioral Profiles of Phosphodiesterase Inhibition in Dopaminergic Neurotransmission.* Journal of Pharmacological Sciences, 2010. **114**(1): p. 6-16.
27. Lipford, M.C. and M.H. Silber, *Long-term use of pramipexole in the management of restless legs syndrome.* Sleep Med, 2012. **13**(10): p. 1280-5.
28. Tan, E.K., *Piribedil-induced sleep attacks in Parkinson's disease.* Fundam Clin Pharmacol, 2003. **17**(1): p. 117-9.
29. DeMarch, Z., et al., *Beneficial effects of rolipram in the R6/2 mouse model of Huntington's disease,* in *Neurobiol Dis*. 2008: United States. p. 375-87.
30. Giampa, C., et al., *Phosphodiesterase type IV inhibition prevents sequestration of CREB binding protein, protects striatal parvalbumin interneurons and rescues motor deficits in the R6/2 mouse model of Huntington's disease,* in *Eur J Neurosci*. 2009: France. p. 902-10.
31. Rajmohan, V. and E. Mohandas, *The limbic system.* Indian J Psychiatry, 2007. **49**(2): p. 132-9.
32. Ramesh, V., et al., *Disrupted sleep without sleep curtailment induces sleepiness and cognitive dysfunction via the tumor necrosis factor-alpha pathway.* J Neuroinflammation, 2012. **9**: p. 91.